

Survival of *Neisseria gonorrhoeae* in an artificial subcutaneous cavity of the mouse

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Man was the only known host susceptible to experimental gonococcal infection until Lucas, Chandler, Martin, and Schmale (1971) succeeded in infecting male chimpanzees by the introduction of gonococcal pus into the urethra and Brown, Lucas, and Kuhn (1972) were able to produce an infection in the chimpanzee using cultured gonococci. More recently, Arko (1972) reported his observations on the fate of gonococci introduced into artificial subcutaneous chambers produced in rabbits, and a small number of similar experiments carried out in guinea-pigs, hamsters, rats, and mice.

Our experiments were planned to provide an opportunity to study *in vivo* the phenomenon reported by Ward, Watt, and Glynn (1970) that gonococci in urethral exudates were resistant to the bactericidal effects *in vitro* of complement plus rabbit and human natural or immune antibodies, although the same strain after repeated subcultures was readily killed by these bactericidal actions.

Material and methods

Swiss Webster mice ranging in weight from 15 to 20 g. were used. They were fed a standard diet and water was given *ad lib*.

Artificial chambers were made in the subcutaneous tissues as follows:

Transparent vinyl tubing of external diameter 12.0 mm. was cut into sections 8 mm. long (Fig. 1) and sterilized by autoclaving (15 lb./sq. in. for 15 min. at 121°C). The hair was removed from the back and loins of the mice, which were then anaesthetized with ether. After the skin had been swabbed with spirit, a transverse incision was made in the skin of the posterior part of the back. The skin was separated from the underlying tissues and the vinyl ring was inserted and pushed towards the head, well away from the incision which was then closed with fine cat-gut sutures (4/0). The stitches were removed after 5 to 6 days and the mice were left for a further 7 days (Fig. 2). At this stage two mice were killed and the tissue

areas covering the vinyl rings were examined macroscopically. They were found to be lined with a layer of granulation tissue to form a small walled-off chamber, containing serous fluid. Microscopic examination of a Leishman-stained film of this fluid revealed the presence of scanty mononuclear cells.

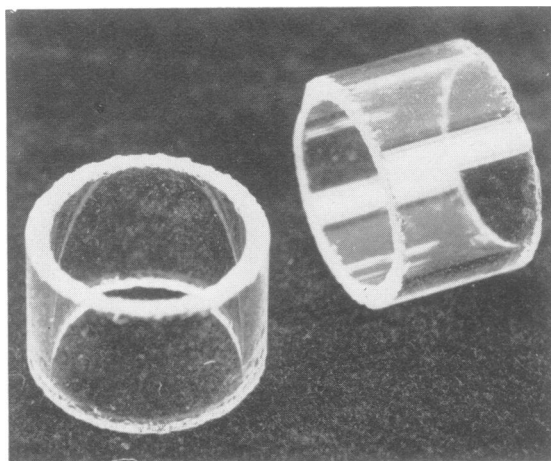


FIG. 1 Vinyl rings $\times 2$



FIG. 2 Artificial cavities in a mouse.

Gonococcal pus was collected from two patients with acute urethritis, suspended in 2 ml. Dulbecco's phosphate buffered saline pH 7.3 and homogenized in a Waring blender to break up the cells present. The viable count of gonococci was then estimated by the method of Miles

and Misra (1938), using a 5 per cent. lysed horse blood agar containing 5 µg./ml. vancomycin. The gonococcal strains isolated from these pus samples were subcultured twice by selective transfer of virulent type I colonies on Difco G.C. medium base (G.C. B. Difco) plus 2 per cent. defined supplement (G.C.M.B.) (White and Kellogg, 1965). Degradation of these strains to avirulent type IV was achieved by unselective subculturing on the same medium (Kellogg, Peacock, Deacon, Brown, and Pirkle, 1963). An old laboratory strain (type IV) was tested in parallel.

Three groups of mice, with one chamber on the left side only, were inoculated with 0.5×10^4 gonococci in 0.2 ml. volumes. The source of the organisms is shown in Table I. Four groups of mice, with chambers on both sides, were inoculated with 0.5×10^5 gonococci in 0.2 ml. volumes. The source of the organisms is shown in Table II.

TABLE I *Source of organisms*

Group	Source of organisms
I	Freshly collected pus (Sample A)
II	Freshly collected pus (Sample B)
III	Type IV laboratory culture

TABLE II *Source of organisms*

Group	Chamber	
	Right	Left
IV	1st subculture of strain A Type I organism	Strain A Type IV organisms
V	1st subculture of strain B Type I organism	Strain B Type IV organisms
VI	2nd subculture of strain A Type I organism	Strain A Type IV organisms
VII	2nd subculture of strain B Type I organism	Strain B Type IV organisms

Results

The mice were observed for 48 hrs and attempts were made to recover gonococci from the chambers at 4, 12, 24, 36, and 48 hrs. This was done aseptically using a syringe with a fine needle to aspirate one or two drops of fluid from the chambers for culture on 5 per cent. lysed horse blood agar and on G.C.M.B. agar. Gonococci were recovered for periods up to 36 hrs from chambers infected with fresh gonococcal pus or with first or second passaged subcultures of type 1. No type IV strains were recovered later than 12 hrs after inoculation, although Gram-staining of the aspirated fluid showed the presence of polymorphs and Gram-negative diplococci.

Discussion

The insertion of these vinyl rings under the skin provides a convenient chamber to study the fate of gonococci in the mouse. It has advantages over colloidon sacs (Harris, 1939) in that it allows both humoral and cellular factors free access to the gonococci.

Our findings suggest that gonococci, both in pus and in freshly-isolated type I colonies, are more resistant to the natural defence mechanisms of the mouse than are type IV strains, but neither type is totally resistant to final destruction by the host's defences. In the mouse chambers we used, the survival of gonococci was very much shorter in time than that observed by Arko (1972). There are, however, differences in experimental procedure in that Arko employed the synthetic steroid dexamethasone (Asuin Schering), and also massive doses of gonococci (approx. 25×10^8) were inoculated into the mice, as opposed to the doses of 0.5×10^4 gonococci/ml. which we used. Further work is clearly required to follow up these findings.

Summary

Artificial chambers, similar to those described by Arko (1972), were created by the introduction of small vinyl rings into the subcutaneous tissues of the mouse. In these chambers we compared the survival of the gonococci from fresh urethral pus with degraded type IV organisms of the same strain after their repeated subculture. We also tested the survival of an established laboratory type IV strain.

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La survie de *Neisseria gonorrhoeae* dans une cavité sous-cutanée artificielle chez la souris

SOMMAIRE

Des chambres artificielles, semblables à celles décrites par Arko (1972) furent réalisées en introduisant de petits

anneaux de vinyl dans le tissu sous-cutané de la souris. Dans ces chambres, nous avons comparé la survie de gonocoques provenant de pus urétral frais avec des organismes de type IV de la même souche dégradés par sub-cultures répétées; nous avons aussi éprouvé la survie d'une souche de collection, stable, de type IV.